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Cryptolepine induced apoptosis in TNFα-stimulated A549 lung carcinoma cells through NF-κB signalling pathway

Olumayokun Olajide[1], Colin Wright[2]. [1]University of Huddersfield, Huddersfield, UK, [2]University of Bradford, Bradford, UK

Cryptolepine, the major alkaloid of the west African shrub *Cryptolepis sanguinolenta*, has been shown to induce cell cycle arrest and apoptosis in A549 cells (Zhu and Godderham, 2006). We have also reported the inhibitory effects of this compound on NF-κB in various cell types (Olajide et al., 2007; 2013a; 2013b). In this study, we have investigated whether the apoptosis-inducing action of the compound is mediated through NF-κB signalling. In order to evaluate the effect on cell proliferation, cultured A549 cells were treated with cryptolepine (5-20 μM) for 24 h, and number of viable cells determined using the MTT assay. Cultured cells pre-treated with cryptolepine (5-20 μM) 30 min prior to stimulation with TNFα (1 nM) were evaluated for levels of caspase 3 using the Caspase-Glo® 3/7 Assay kit (Promega). The effects of cryptolepine on TNFα-induced IκB phosphorylation, NF-κBp65 subunit nuclear translocation, and protein expressions of NF-κB-regulated gene products of apoptosis (cyclin D1, survivin, XIAP, cIAP1, and Bcl-2 were investigated by treating cultured A549 cells with cryptolepine (5-20 μM) 30 min before stimulation with TNFα (1 nM), followed by In Cell western analysis. Results showed that cryptolepine produced dose-dependent and significant ($p<0.05$) reduction in A549 cell proliferation after 24 h of treatment. At 20 μM of the compound, cell viability was reduced by 62.2±3.3%. Treatment with 10 and 20 μM cryptolepine for 24 h was also found to cause significant ($p<0.05$) induction of caspase-3. With 10 μM, relative luminescence was 9038±480.5, and at 20 μM, relative luminescence was 9776±266.4, compared with relative luminescence of 1151±74.5 recorded in control cells. Protein analyses revealed that 10 and 20 μM of cryptolepine inhibited TNFα-induced IκB phosphorylation and NF-κBp65 nuclear translocation. Cells stimulated with TNFα (1 nM) showed elevated levels of Bcl-2, cyclin D1, surviving, XIAP and cIAP, which were reduced when pre-treated with cryptolepine (5-20 μM). Our results showed that cryptolepine downregulated the expression of anti-apoptosis proteins. We have also demonstrated that cryptolepine induces apoptosis in A549 lung carcinoma cells by interfering with NF-κB signalling.

References

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